1.35-3.15 μm·s−1·HSL−1) (n = 6 half-sarcomeres; n = 13 single sarcomeres) during activation show an increase in force during and after stretch, which was long lasting (i.e. residual force enhancement). Despite a high variability, half-sarcomeres showed a tendency to produce a smaller level of residual force enhancement when compared to single sarcomeres. Our results show that half-sarcomeres generate levels of isometric force that are similar to those observed in sarcomeres and myofibrils. However, half-sarcomeres produced a lower level of stretch-induced force enhancement when compared to sarcomeres.

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N-Terminal Truncation of Flightin Reduces Oscillatory Power Output without Affecting Cross-Bridge Kinetics in Drosophila Indirect Flight Muscles

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The asynchronous, indirect flight muscles (IFM) of *Drosophila* are characterized by a high passive stiffness and exceptionally fast myosin kinetics, two attributes that enhance power output to sustain flight. Flightin is an IFM-specific, 20kDa myosin rod-binding protein required for normal thick filament stiffness, sarcomere integrity, and flight. Previously, we showed that a COOH-terminal truncation of flightin ($fln^{DeltaC44}$) decreased myofilament lattice order and myosin kinetics, resulting in lower oscillatory power output and flightlessness. Here, we investigate the function of the flightin N-terminal 62 amino acids by creating transgenic *Drosophila* ($fln^{DeltaN62}$) expressing a truncated flightin. $fln^{DeltaN62}$ flies were flight impaired (flight index: 2.8 ± 0.1 vs. 4.2 ± 0.4 for $fln^{DeltaN62}$ vs. fln^{+} rescued null control) despite having a normal wing-beat frequency (195 ± 4 vs. 198 ± 2 Hz for fln^{+}). Mechanical analysis of skinned IFM fibers showed that the flightin N-terminal truncation reduced passive, active, and rigor stiffness without affecting cross-bridge kinetics (frequency of maximum power: 205 ± 7 vs. 217 ± 7 Hz for fln^{+}). $fln^{DeltaN62}$ fibers produced approximately half the isometric tension (passive: 0.9 ± 0.1 vs. 1.7 ± 0.3 kN/m^2, active: 0.8 ± 0.1 vs. 1.5 ± 0.2 kN/m^2, rigor: 1.1 ± 0.2 vs. 3.1 ± 0.4 kN/m^2) and maximum oscillatory power output (38.0 ± 4.6 vs. 89.5 ± 9.6 W/m^3) as fln^{+} fibers. Moreover, about 60% of the $fln^{DeltaN62}$ fibers tore in rigor, demonstrating mechanical failure near isometric tension values that were sustained by fln^{+} fibers. Fourier transform analysis of cross-sectional electron micrographs revealed that the flightin N-terminal truncation compromised myofilament lattice crystallinity and reduced inter-thick filament spacing by 10% (44.1 ± 1.3 vs. 49.7 ± 0.4 nm). These results indicate that the flightin N-terminal region enhances myofilament lattice order and mechanical integrity, which in turn is required for effective force transmission, normal oscillatory power output, and flight.

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A New Functional Measure of Contractility in Human Cardiomyopathies

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Hypertrophic and familial dilated cardiomyopathies are arguably the most common forms of inherited myocardial dysfunction. Both disease states result in deterioration of cardiac function and quality of life as a consequence of extensive remodelling of the chamber walls.

Here we use a novel technique SPontaneous Oscillatory Contractions - or SPOCs to assess changes in contraction and relaxation phases using a range of explanted human heart samples. We examine left ventricle (LV) samples from: (1) patients with hypertrophic cardiomyopathy (HCM); (2) patients with familial dilated cardiomyopathy (FDCM); and (3) and aged-matched non-failing donors. The SPOC parameters of interest are: (i) SPOC amplitudes; (ii) the rates of lengthening (relaxation); (iii) the rates of shortening (contraction); and (iv) their respective SPOC periods.

On average, samples from HCM patients exhibited significantly slower rates of lengthening and shorter SPOC periods, while FDCM patients displayed significantly longer lengthening and shortening SPOC periods, compared to donors. Impaired shortening is indicative of diastolic dysfunction while impaired shortening indicates a systolic dysfunction. We observed extensive changes in SPOC parameters that were mutation-specific. The MYBPC3 mutation exhibited shorter SPOC period and faster shortening rates while the samples with the TNNI3 mutation had a higher amplitude and slower shortening rates. Their SPOC data are consistent with the relatively mild phenotype associated with their respective mutations. Furthermore, SPOC is also sensitive to the progressive deterioration in LV ejection fraction.

SPOC analysis is a promising tool that provides a quantitative insights into cardiac contractility. It may allow us to unravel other significant differences between familial cardiomyopathies and donor hearts. The SPOC data agree well with patient clinical phenotypes.

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Full Tropomyosin Movement during Stretch Activation Requires Strong Binding Crossbridges

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Using real-time X-ray diffraction movies of cyclically stretch-activated IFM, we previously established that stretch activation (**SA**) is controlled by tropomyosin (**TM**), similar to the well-known calcium-activated steric blocking mechanism, but mechanically triggered by myosin-troponin connections. Here, we probed whether TM controls SA via the same three-state mechanism that is the standard model for calcium-activation, in which TM adopts three positions, called Blocked (pCa ~9), Closed (pCa ~4.5), and Open (with strong myosin binding). The key feature of the three-state mechanism is that calcium binding to troponin only moves TM to the Closed position, which partially reveals actin's myosin-binding sites and allows weak actin-myosin contact; strong-binding myosin heads are required to nudge TM aside to the fully Open position. We used vanadate to block strong binding by myosin during X-ray diffraction movies of fibers length-cycled in the presence of vanadate and Ca^{2+}. The reflection that reports TM movement (outer part of the 19.3-nm layer line) indicates a partial TM movement, as compared to un-inhibited fibers that are actively contracting. Thus SA appears to be a variation of the three-state mechanism seen during calcium-activation, except that the sub-saturating [Ca^{2+}] typical of SA (pCa ~6) leaves TM in the Blocked position until sarcomere stretch moves TM to the Closed position. Regardless of whether the fiber is stretch-activated at pCa 6, or isometrically Ca^{2+}-activated at pCa 4.5, it still requires strong-binding myosin heads to nudge TM to the fully Open position. Other changes seen in the X-ray movies of vanadate-inhibited fibers are consistent with our previous interpretation that myosin-troponin bridges mediate TM movement during sarcomere stretch, and indicate that myosin-troponin bridges are not affected by vanadate.

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Factors Beyond Detachment Kinetics that Influence Unloaded Shortening Velocities of Muscle

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Recent studies demonstrate the influence of actin-myosin attachment kinetics on in vitro actin filament sliding velocities (V), but it is unclear what factors determine whether velocities are detachment (d/τ_{on}) or attachment limited (Yengo et al. 2012. *J Muscle Res Cell Motil.*). We have developed a muscle model with molecular detail that suggests three key mechanisms by which attachment kinetics can influence unloaded shortening velocities. First, the saturation of myosin binding sites on actin by weakly bound myosin heads can result in attachment limited velocities that are slower than the detachment limit. Essentially, at high myosin densities the actin-activated ATPase activity (attachment limited) in the motility assay saturates before mechanical saturation (detachment limited). Second if actin-bound myosin heads that resist actin movement have a relatively low stiffness, unloaded shortening velocities can exceed the detachment limit. The non-linear compliance of positively and negatively strained myosin heads was demonstrated experimentally (Kaya and Higuchi. 2010. *Science*. 329: 686-9). Finally, our model shows that mechanically accelerated ADP release kinetics can result in velocities that exceed the detachment limit. These simulations accurately describe the observation of hypermotile velocities (Hooft et al. 2007. *Biochemistry.* 46:3513-20., Jackson and Baker. 2009. *PCCP*. 11:4808-14.). In summary, unloaded shortening velocities can be influenced by many factors other than detachment kinetics. Thus a mutation or perturbation that results in a change in V need not result from a change in only d or τ_{on}.

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Variable Stabilities Along the Myosin Coiled Coil Revealed by Free Fall Force Spectroscopy

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